2. **Mutagenicity:**

In 1991, the Carcinogenicity Peer Review Committee concluded that there was no evidence of genotoxicity for glyphosate based on negative findings in submitted guideline studies for the bacterial reverse mutation test (MRID 00078620), *in vitro* mammalian cell gene mutation test in CHO cells (MRID 00215737), *in vivo* mammalian erythrocyte micronucleus test (MRID 0025137) and in a "rec assay" used to detect DNA damaging agents in *Bacillus subtilis* (MRID 00078619) (TXR# 0008898). Glyphosate was also negative in two unacceptable studies evaluating DNA repair in rat hepatocytes (MRID 00075619) and dominant lethal mutations in mice (MRID 00057072).

Glyphosate has also been evaluated for its genotoxic potential in other regulatory and published literature studies. Extensive reviews of the available genotoxicity studies for glyphosate and glyphosate products were conducted by Williams *et al.* (2000) and by Kier and Kirkland (2013). IARC also conducted a review of the publically available genetic toxicity data for glyphosate and glyphosate-based formulations (IARC Monograph, 2015).

Williams *et al.*, (2000) concluded that "glyphosate is neither mutagenic nor clastogenic." Similarly, Kier and Kirkland concluded a "lack of genotoxic potential for both glyphosate and glyphosate based formulations (GBFs) in core gene mutation and chromosomal effect endpoints." Kier and Kirkland (2013) also stated that "the observations of DNA damage effects seems likely to be secondary to cytotoxic effects". However, IARC (2015) concluded that "there is strong evidence that glyphosate cause's genotoxicity". It should be noted that the IARC's conclusion was based not only on studies conducted with the active ingredient but also on studies conducted with the formulation products such as Roundup. Round up is a combination of the active ingredient and other chemicals, including a surfactant (polyoxyethyleneamine) which enhances the spreading of spry droplets when contact foliage. Also, review article by Kier and Kirkland and supplemental information provided on the publisher's website were not considered in the IARC evaluation.

In this assessment, the CARC considered the studies submitted to the Agency under 40 CFR Part 158 as well as the studies presented in the review articles by Williams *et al.* (2000), Kier and Kirkland (2013) and the IARC monograph (2015). Consistent with OPP's Guidance for Considering and Using Open Literature Toxicity Studies to Support Human Health Risk Assessment (http://www.epa.gov/pesticides/science/lit-studies.pdf), literature studies discussed in the reviews such as IARC that did not meet the Klimisch criteria for reliability (*e.g.* lack of inadequate glyphosate purity information or the test material) were not considered by CARC. CARC determined the mutagenic potential of glyphosate in humans by conducting a weight of evidence evaluation of the results from the cited bacterial reversion (Ames) assays, *in vitro* mammalian gene mutation assays, *in vitro* and *in vivo* chromosomal aberration and micronucleus assays as well as other relevant assays evaluating DNA damage.

a. Bacterial reverse mutation assays

As shown in Table 15, glyphosate was not mutagenic in any of the *in vitro* bacterial mutation assays using *S. typhimurium* or *E. coli* tester strains with or without microsomal S9 metabolic activation. These results are consistent with the negative findings in the previously reviewed EPA guideline (870.5100) bacterial reverse gene mutation study (MRID 00078620).

Table 15 Results from Bacterial Reverse Gene Mutation Assays[1] 1.

Author	Cell/Strain[2]	Purity	Highest test concentration	Results –S9	Results +S9
Akanuma, M. (1995)	TA98, TA100, TA1535, TA1537; WP2*uvr*A	95.7%[3]	5000 µg/plate	Negative	Negative
Callander, R.D. (1996)	TA98, TA100, TA1535, TA1537; WP2P and WP2*uvr*A	95.6%[3]	5000 µg/plate	Negative	Negative
Flügge, C. (2010)	TA98, TA100, TA102, TA1535, TA1537	76.1%[4]	100 µg/plate	Negative	Negative
Flügge, C. (2010)	TA98, TA100, TA102, TA1535, TA1537	96.4%	3160 µg/plate	Negative	Negative
Flügge, C. (2009)	TA98, TA100, TA102, TA1535, TA1537	98.8%	3160 µg/plate	Negative	Negative
Jensen, J.C. (1991)	TA98, TA100, TA1535, TA1537	98.6%	2500 µg /plate w/o S9; 5000 µg /plate w/ S9	Negative	Negative
Li and Long (1998)	TA98, TA100, TA1535, TA1537, TA1538;	98%	5000 µg/plate	Negative	Negative
NTP (1992)	TA97, TA100, TA1535	98%	10,000 µg /plate	Negative	Negative
Schreib, G. (2010)	TA98, TA100, TA1535, TA1537; WP2*uvr*A	96%	5000 µg/plate	Negative	Negative
Shirasu et al. (1978)	TA98, TA100, TA1535, TA1537, TA1538 and WP2*uvr*A	98.4%	5000 µg/plate	Negative	Negative
Sokolowski, A. (2007c)	TA98, TA100, TA1535, TA1537; WP2*uvr*A	95.0%	5000 µg/plate	Negative	Negative
Sokolowski, A. (2007a)	TA98, TA100, TA1535, TA1537; WP2uvrA	95.1%	5000 µg/plate	Negative	Negative
Sokolowski, A. (2009b)	TA98, TA100, TA1535, TA1537;WP2P and WP2*uvr*A	96.3%	5000 µg/plate	Negative	Negative
Sokolowski, A. (2009a)	TA98, TA100, TA1535, TA1537; WP2*uvr*A	96.66%	5000 µg/plate	Negative	Negative
Sokolowski, A. (2007b)	TA98, TA100, TA1535, TA1537; WP2*uvr*A	97.7%	5000 µg/plate	Negative	Negative
Suresh, T.P. (1993)	TA98, TA100, TA1535, TA1537, TA1538	96.0%	1000 µg/plate	Negative	Negative
Thompson, P.W. (1996)	TA98, TA100, TA1535, TA1537; WP2*uvr*A	95.3%	5000 µg/plate	Negative	Negative

Studies cited in Williams *et al*., (2000), Kier and Kirkland (2013), or IARC monograph.
2. *Salmonella typhimurium* strains (TA97, TA98, TA100, TA102, TA1535, TA1537, and/or TA1538) or *E. coli* strains (WP2P and WP2*uvr*A)
3. Glyphosate acid
4. Monoammonium glyphosate salt

b. *In vitro* mammalian cell gene mutation assays

Glyphosate did not induce forward mutations in mouse lymphomas cells or Chinese hamster ovary (CHO) cells in the presence or absence of metabolic (S9) activation (Table 16).

Table 16. Results from mammalian gene mutation assays[1].						
Author	**Assay Type**	**Cell type**	**Purity**	**Highest conc.**	**Result -S9**	**Result +S9**
Clay (1996)	*In vitro* mammalian gene mutation	L5178Y mouse lymphoma cells/ tk locus	95.6%	1.0 mg/mL	Negative	Negative
Jensen, J.C. (1991)	*In vitro* mammalian gene mutation	L5178Y mouse lymphoma cells/ tk locus	98.6%	5.0 mg/mL	Negative	Negative
Li and Long (1988)	*In vitro* mammalian gene mutation	CHO cells/ HGPRT locus	98%	22.5 mg/mL	Negative	Negative

1. Studies cited in Williams *et al*., (2000), Kier and Kirkland (2013), or IARC monograph.

c. *In vitro* chromosomal aberration assays

Lioi *et al*., reported positive findings for chromosomal aberrations in human and bovine lymphocytes treated with glyphosate *in vitro* in the absence of S9 activity. However, Van de Waart reported no significant increase in chromosomal aberrations in human lymphocyte treated with up to 0.56 mg/mL (-S9) and 0.33 mg/mL (+S9) glyphosate, an approximately 70-fold higher concentration than where Lioi *et al*. reported aberrations. Glyphosate was negative in two other *in vitro* chromosomal aberrations studies in human lymphocytes (Fox, 1998 and Manas, 2009) and did not induce chromosomal aberrations in Chinese hamster lung cells (Matsumoto, 1995 and Wright 1996). A summary of the findings is presented in Table 17.

Table 17. Results from *in vitro* chromosomal aberration assays[1].						
Authors	**Assay**	**Cell type**	**Purity**	**Highest test concentration**	**Result -S9**	**Result +S9**
Van de Waart (1995)	Chromosomal Aberration	Human peripheral lymphocytes	>98%	0.56 mg/mL with S9; 0.33 mg/mL w/o S9	Negative	Negative
Fox, V. (1998)	Chromosome Aberration	Human peripheral lymphocytes	95.6%[2]	1250 ug/mL	Negative	Negative
Lioi et al. (1998a)	Chromosomal Aberration	Human peripheral lymphocytes	>98%	1.4 mg/L	Positive	Not Tested
Manas et al. (2009)	Chromosomal Aberration	Human peripheral lymphocytes	96%	6 mM	Negative	Not Tested
Lioi et al. (1998b)	Chromosomal Aberration	Bovine peripheral lymphocytes	>98%	2.9 mg/L	Positive	Not Tested
Matsumoto, K. (1995)	Chromosomal Aberration	Chinese Hamster Lung (CHL) cells	95.68%[2]	1000 ug/mL	Negative	Negative
Wright, N.P. (1996)	Chromosomal Aberration	Chinese Hamster Lung (CHL) cells	95.3%	1250 ug/mL	Negative	Negative

1. Studies cited in Williams *et al*., (2000), Kier and Kirkland (2013), or IARC monograph.
2. Glyphosate acid

d. *In vivo* micronucleus and chromosomal aberration assays

Numerous studies were evaluated to determine the potential for glyphosate to induce micronuclei in rodent bone marrow cells. Studies included both intraperitoneal (IP) and oral routes of glyphosate administration. In a literature study by Bolognesi *et a*l. (1997), the authors reported an induction of micronuclei in male mice treated with up to 300 mg/kg (injected as two ½ doses). It is noted that this study included only 3 animals/dose; rather than the 5 animals/dose recommended in the agency's test guideline (870.5395). In another literature study, Manas *et a*l. (2009) reported an induction of micronuclei in BALB/C mice when tested up to 200 mg/kg glyphosate. Additionally, Suresh *et al*. (1993) reported an increase in micronuclei in females only in Swiss albino mice treated with 5 mg/kg glyphosate; a dose that is more than twice the limit dose for the agency's guideline study. Although the above authors reported positive findings, a vast majority of the *in vivo* genotoxicity studies (including the previously reviewed guideline mammalian micronucleus test) were negative at doses similar to or higher than the studies discussed above, regardless of the dosing regimen or route of administration. A summary of the findings are reported in Table 18.

Table 18. Results from *in vivo* genotoxicity assays[1].

Author	Assay Type	Species/strain	Purity	Highest conc.	Results	Comments
Bolognesi *et al.* (1997)	Micronucleus test	Male mice (strain not provided)	99.9%	300 mg/kg	Positive	Two IP injections of ½ dose; 3 mice/dose
Durward, R. (2006)	Micronucleus test	Young adult male and female albino Crl:CD - 1TM(ICR)BR mice	95.7%	600 mg/kg	Negative	Single IP injection; Significant increase in % PCEs per 1000 erythrocytes was observed in the 24-hour; however not 48 - hour at 600 mg/kg
Flügge, C. (2009)	Micronucleus test	Male and female CD rats	98.8%	2000 mg/kg	Negative	Single dose; oral gavage
Fox and Mackay (1996)	Micronucleus test	Male and female CD-1 BR mice	95.6%[2]	5000 mg/kg	Negative	Single dose; oral gavage
Honavar, N. (2005)	Micronucleus test	Male and female NMRI mice	97.73 %	2000 mg/kg	Negative	Single dose; oral gavage
Honavar, N. (2008)	Micronucleus test	NMRI male mice	99.1%	2000 mg/kg	Negative	Single dose; oral gavage
Jensen, J.C.	Micronucleus	Young adult male	98.6%	5000	Negative	Single dose; oral

(1991)	test	and female NMRI SPF mice		mg/kg		gavage
Manas et al. (2009)	Micronucleus	BALB/C mice	96%	200 mg/kg	Positive	Two IP doses, 1 day apart
NTP (1992)	Micronucleus test	Male and female B6C3F1 mice	99%	11,379 mg/kg/day	Negative	Dietary admin., 13 weeks
Suresh, T.P. (1993)	Micronucleus test	Young Swiss albino male and female mice	98.6%	5000 mg/kg	Males: Negative. Females: Positive	Two doses 1 day apart; oral gavage
Suresh, T.P. (1994)	Mouse Bone Marrow Chromosome Aberration	Male and female Swiss albino mice	96.8%	5000 mg/kg	Negative	Two doses, 24 hours apart; oral gavage

1. Studies cited in Williams *et al*., (2000), Kier and Kirkland (2013), or IARC monograph.
2. Glyphosate acid
3. IP= intraperitoneal injection

e. Other genotoxicity assays

Inconsistent responses were reported in number of assays designed to detect DNA damage, including sister chromatid exchange (SCE) assay, unscheduled DNA synthesis assay, and the comet assay (also known as the single cell electrophoresis assay). Positive responses in these assays do not necessarily indicate a chemical is DNA-reactive (*i.e.* mutagenic), but rather under conditions of the assay, DNA damage occurred. Glyphosate was negative in two rodent dominant lethal test and in two Rec- DNA repair tests in *B. subtilis*. The results of these genotoxicity studies are presented in Table 19.

Table 19. Additional genotoxicity assays

Authors	Assay Type	Cell Type	Purity	Highest test conc.	Results
Bolognesi et al. (1997)	Sister chromatid exchange (SCE)	Human Peripheral blood (in vitro)	99.9%	1000 ug/mL	Positive
Lioi et al. (1998a)	SCE	Human Peripheral blood (in vitro)	>98%	1.4 mg/L	Equivocal
Lioi et al. (1998b)	SCE	Bovine Peripheral blood (in vitro)	>98%	2.9 mg/L	Equivocal
Li and Long (1988)	Unscheduled DNA synthesis (UDS)	Rat hepatocytes (in vitro exposure)	98%	0.125 mg/mL	Negative
Rossberger, S. (1994)	UDS	Primary rat hepatocytes	98%	111.69 mM	Negative
Bolognesi et al. (1997)	DNA Damage/reactivity/ UDS	Mouse; IP administration	99.9%	300 mg/kg	Equivocal
Bolognesi et al. (1997)	DNA Damage/reactivity/ UDS	Mouse; IP administration; alkaline solution of extracted DNA	99.9%	300 mg/kg	Positive
Alvarez-Moya et al. (2014)	Comet assay	Human lymphocytes	96%[2]	700 µM	Positive

Lueken et al. (2004)	Comet assay	Human fibroblasts GM 5757	98.4%	75 mM	Negative
Manas et al. (2009)	Comet assay	Liver Hep-2 cells	96%	7.5 mM	Positive
Mladinic et al. (2009)	Comet assay	Human lymphocytes	98%	580 ug/mL (toxic); approx 3.43 mM	Positive
Rossberger, S. (1994)	DNA repair test	Male SD rat primary hepatocytes	>98%	111.69 mM	Negative
Suresh, T.P. (1992)	Rodent dominant lethal test	Male and female Wistar rats	96.8%	500 mg/kg (single dose); 100 mg/kg (5 daily doses)	Negative
Wrenn (1980)	Rodent dominant lethal test	Mouse; gavage	98.7%	2000 mg/kg	Negative
Akanuma, M. (1995)	DNA repair test (Rec- assay)	*Bacillus subtilis* M45 rec-/ H17 rec+	95.68% [2]	240 ug/disk	Negative
Li and Long (1988)	DNA repair test (Rec assay)	*B. subtilis* H17, rec+; M45, rec-	98%	2 mg/disk	Negative

1. Studies cited in Williams *et al.*, (2000), Kier and Kirkland (2013), or IARC monograph.
2. Glyphosate acid

f. Conclusions

In summary, glyphosate was not mutagenic in bacteria or mammal cells *in vitro*. Additionally, glyphosate did not induce chromosomal aberrations *in vitro*. Although some studies in the open literature reported positive findings for micronuclei induction in rodents, these findings were not replicated in the majority of the rodent micronuclei studies considered in this assessmnt by CARC. Some positive results were reported SCE and comet assays in the open; however, there is no convincing evidence that the DNA damage is a direct effect of glyphosate, but rather may be a secondary to cytotoxicity or oxidative damage.